Double-beam near-infrared spectroscopy to correct light source drift in aqueous glucose solution experiments

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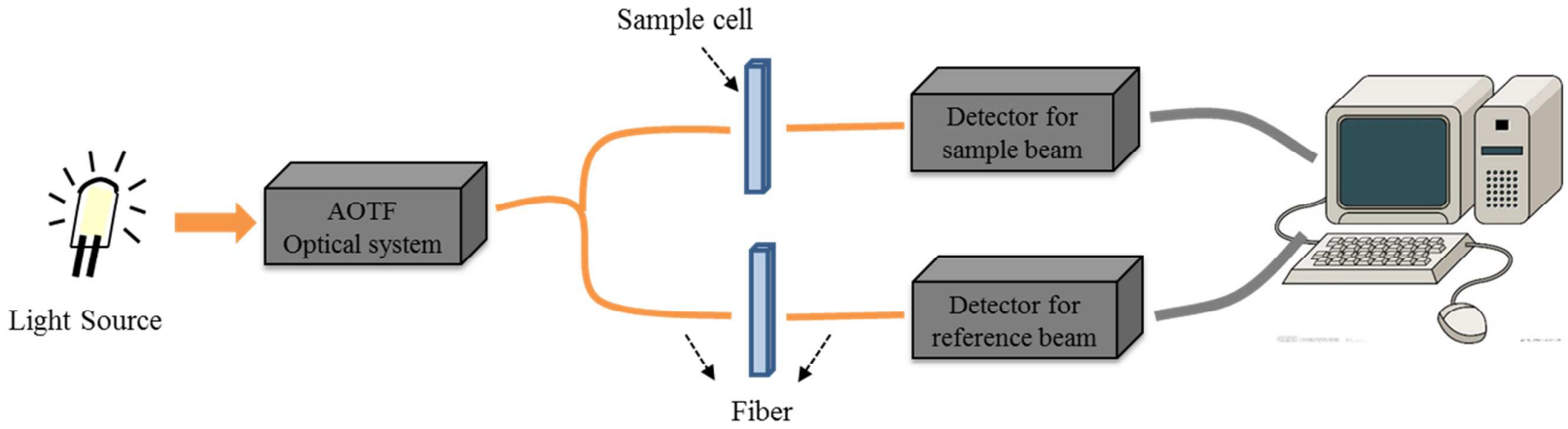
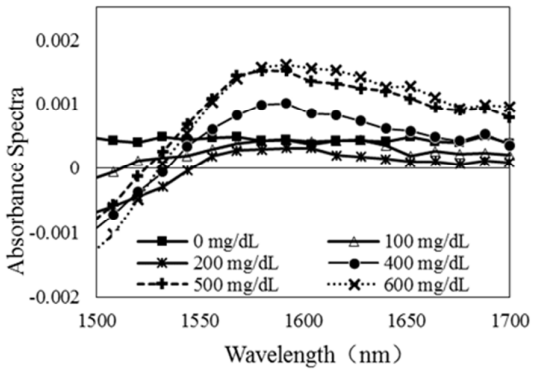
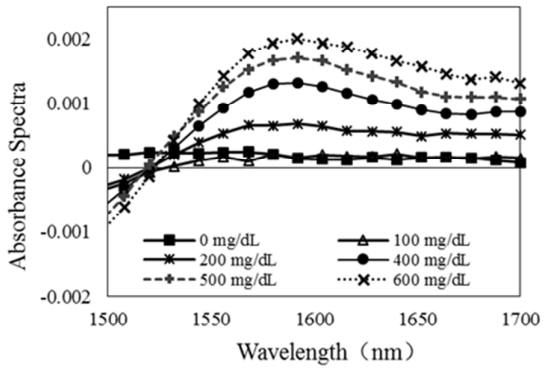
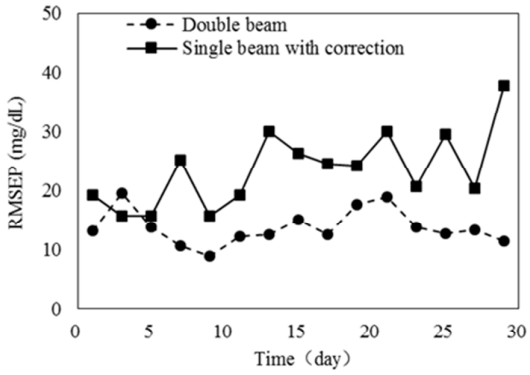
Standard sample correction and reference design for exact extraction of the analyte interested are investigated in this paper.









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Double-beam near-infrared spectroscopy to correct light source drift in aqueous glucose solution experiments

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Currently, a key problem in near-infrared (NIR) non-invasive blood glucose sensing is that the influence of the background changes during measurements, which restricts the effective extraction of unique glucose information. As the influence of the background can only be reduced by various correction techniques, standard sample correction and reference beam designs are investigated in this paper. First, the basic principles and preconditions for the background correction of single-beam measurements with reference correction and double-beam measurements with double detectors are analyzed. Second, the signal-to-noise ratio (SNR) under different measurement modes is evaluated. Third, single-day and multi-day experiments are conducted on aqueous glucose solution. The results are as follows: (1) Short time interval between measurements of sample and reference is the prerequisite for reference correction measurement with single-beam. The double-beam measurement has no special requirement for the SNR and the sampling time interval. (2) The double-beam design is more effective at eliminating the time-dependent variations. The SNR of the single-beam measurement can be improved by correcting the reference sample, which is also verified by the 2D correlation spectroscopy analysis under the perturbation of time. (3) The predictive ability of the partial least squares (PLS) model based on double-beam measurement is the best for both single-day experiments and multi-day experiments, which is consistent with the result of SNR test. Both the reference sample correction and the reference beam design can effectively reduce the light source drift, and the double-beam design is more appropriate for multi-day or long-term experiments.

1. Introduction

Diabetes is a common chronic, non-infectious disease that may lead to serious damage to various organs over time, particularly the nerves and blood vessels. According to a WHO report from 2013 [1], 347 million people worldwide have diabetes. As there is no medical cure for diabetes, WHO recommends that patients perform self-monitoring of blood glucose (SMBG) frequently to reduce the risk of the complications. However, the traditional blood glucose test is invasive because the finger is pricked to obtain blood, and this restricts the frequency of SMBG and decreases the quality of life.

In the past 30 years, many methods have been developed to measure blood glucose noninvasively [2-10]. The near-infrared spectroscopy technique is one of the most promising methods, [11-12] but its accuracy is restricted by the background fluctuations during the measurement process. Even a small background change may lead to significant spectral variations and can thus obstruct the exact extraction of the analyte of interest. In this case, the calibration model will be unstable and will have lower predictive ability. Many researchers have applied background correction methods to reduce the influence of background variations. Arnold *et al.* conducted *in vitro* aqueous solution experiments in the first-order overtone band of glucose; spectra of pure water were collected as the references after each set of three glucose samples. A standard error of prediction (SEP) of 1.28 mM (23.04 mg/dL) was achieved for the calibration model after removing high-frequency noise with a digital Fourier filter algorithm [13]. Heise *et al.* also measured the glucose levels in plasma samples with water as the reference, and the SEP value of PLS models were 16.3 mg/dL and 18 mg/dL over the first-order overtone and the combination band, respectively [14]. Ozaki *et al.* measured the diffuse reflectance spectra of the human forearm *in vivo* over the wavelength range of 1200 to 1900 nm, for which a diffuse reflectance standard was chosen as the reference. The final SEP ranged from15.3 to 32.4 mg/dL [15-16]. In the above correction methods, a stable standard sample was selected as the reference to

reduce the short-term systematic drift, and high-quality results were obtained.

In the NIR spectrum, the background fluctuations mainly come from two sources: the instrument and the sample for analysis. To take into account the influence of instrument fluctuations and sample change due to the temperature and humidity, *etc*, in a single-beam measurement, a standard sample that has similar optical characteristics to those of sample for analysis is usually selected as the reference. This method is defined as a single-beam measurement with reference correction. Another possible way to reduce the background fluctuation is a double-beam measurement, where one reference beam is designed to record and correct the systemic drift. According to previous research, the premise of a single-beam measurement is to minimize the time interval between measurements of the reference and the sample. However, such time intervals cannot be reduced to a satisfactory level due to the complexity of the sample and spectrum acquisition, making this method unsuitable for an elaborate *in vivo* experiment with constantly varying physiological status, which further limits the application of a single-beam measurement. Conversely, a double-beam measurement overcomes the shortcoming of single-beam measurement caused by time intervals of the measurement. However, this method may still be affected by differences between the two detecting circuits and detectors. Because both methods have their own advantages and disadvantages, it is necessary to systematically compare the extent to which each of the methods can be applied.

In this paper, we analyze the principles of single-beam and double-beam measurement modes and their correction mechanisms for spectral variation due to background fluctuations. Based on a set of assumptions, *i.e.,* the time intervals of every two adjacent measurements remain constant during the measurement and the intensity of incident light drifts towards the same direction, an argument is adopted to describe the drift level of the light source to visually view the correction effects. Single-beam and double-beam systems are designed to conduct experiments on aqueous glucose solutions to further validate the correction theory. Finally, the instrument performance and the correction effect for the instrumental drift, mainly the light source drift, from both single-day and multi-day experiments are evaluated, and glucose-specific information are extracted to build the calibration model.

2. Theory

The interference of background fluctuations during measurements is one of the most difficult problems in NIR spectroscopy analysis. Practically, background changes due to the instrumental hardware mainly contains two sources: 1) inherent noise in the system, such as thermal noise and shot noise, and 2) drift from the instrument during the measurement, such as baseline drift caused by variation in the light intensity and wavelength shift caused by changes in the detector performance. Despite the advanced hardware in the modern spectrometer, appropriate data processing methods are still needed because there is no rule for the direction or amplitude of instrumental drift. Once the power to the light source or detectors is on, the drift occurs. Usually, the SNR of the spectrometer decreases with increasing measurement time.

2.1 Single-beam measurement

Based on the Beer-Lambert law, the light intensity transmitted through the sample is:

$$I(t) = I_0(t)e^{-\sum_i \varepsilon_i c_i l} \quad (1)$$

where t is time, $I_0(t)$ is the intensity of the incident light, $I(t)$ is the intensity of the transmitted light, i is the number of components in the sample, ε and c are molar absorptivity and concentration of each component of the sample, and l is the optical path length. The transmitted light can also be expressed in terms of an absorbance:

$$A = \ln\frac{I_0(t)}{I(t)} = \ln\frac{1}{T} \quad (2)$$

where A and T are the absorbance and transmittance of the sample, respectively. The incident light intensity $I_0(t)$ drifts over time. In NIR spectroscopy analysis, the absorbance differences of various samples are usually calculated instead of measuring $I_0(t)$ directly.

Taking an aqueous glucose solution as an example, the absorbance difference between samples *j* and *k* can be expressed as:

$$\Delta A(g) = \ln\frac{I(t_{sk})}{I(t_{sj})} = \ln\frac{I_0(t_{sk})\cdot e^{-(\sum_i \varepsilon_i \cdot C_i \cdot l + \varepsilon_g \cdot C_{gk} \cdot l)}}{I_0(t_{sj})\cdot e^{-(\sum_i \varepsilon_i \cdot C_i \cdot l + \varepsilon_g \cdot C_{gj} \cdot l)}} = \ln\frac{I_0(t_{sk})}{I_0(t_{sj})} + \varepsilon_g \cdot \Delta C \cdot l \quad (3)$$

where i is the number of components in the sample except glucose, ε_g is the molar absorptivity of glucose, C_{gj} and C_{gk} are concentrations of glucose in sample *j* and *k* respectively, and $\Delta C = C_{gj} - C_{gk}$.

Here, the absorbance difference between two samples in the single-beam measurement includes information about the incident light intensity. If the measurement interval between two samples is quite long or the spectra of the two samples are collected on different days, the absorbance difference will be not linear with the concentration difference between samples.

To reduce the influence from the fluctuations of I_0, a standard sample is often used, *i.e.*, a single-beam measurement with reference correction, where the spectra of the standard sample and the analyzing sample were collected alternately by the same detector. Then, the equivalent absorbance of the sample, A_e, can be expressed as:

$$A_e = \ln\frac{I_b(t_b)}{I_s(t_s)} = \ln\frac{I_{0b}(t_b)\cdot T_b}{I_{0s}(t_s)\cdot e^{-(\sum_i \varepsilon_i \cdot C_i \cdot l + \varepsilon_g \cdot C_g \cdot l)}} \quad (4)$$

where I_s and I_b are the intensity of the transmitted light through the sample and the standard, respectively, and T_b is the transmittance of the standard. After the correction of corresponding reference, the absorbance difference between samples *j* and sample *k* is:

$$\Delta A(g) = A_{ej} - A_{ek} = \ln\frac{I_{0bj}(t_{bj})}{I_{0sj}(t_{sj})}\cdot\frac{I_{0sk}(t_{sk})}{I_{0bk}(t_{bk})} + \ln\frac{T_{bj}}{T_{bk}} + \varepsilon_g \cdot \Delta C_g \cdot l \quad (5)$$

Assuming that the short-term SNR of the spectrometer is high enough, if the measurement interval between the sample and the corresponding reference is short, *i.e.*, $t_b = t_s$, the incident light

intensities can be considered to be constant, *i.e.*, $I_{0s}(t_s) = I_{0b}(t_b)$. Then, eq. 5 can be rewritten as:

$$\Delta A(g) = \ln \frac{T_{bj}}{T_{bk}} + \varepsilon_g \cdot \Delta C_g \cdot l \qquad (6)$$

Only if the optical characteristics of the reference sample are stable during the measurement process, that is, $T_{bj} = T_{bk}$, the absorbance difference is linear with the glucose concentration change, which is quite important for further linear regression analysis. In other words, the drift of the light source during the experiment is monitored and compensated effectively by taking advantage of the reference sample. However, high short-term instrument SNR and a short sampling interval are required, and more acquisition time is needed in this time-sharing measurement mode due to switching the sample and reference frequently.

Here, the relative change of light intensity, σ, is used to descript the drift level:

$$\sigma = \frac{\Delta I}{I_0} \qquad (7)$$

where I_0 and ΔI are the initial incident light intensity and its relative variation in the time interval of two adjacent measurements (Δt). Obviously, the smaller the value of σ is, the smaller the drift will be.

It is critical to note that the following derivations are based on a set of assumptions: (1) during the experiment, the time intervals of every two adjacent measurements remain constant; (2) the intensity of I_0 drifts in the same direction; and (3) samples *j* and *k* are two adjacent measured samples.

In a single-beam measurement, if sample *j* is measured first, the absorbance difference between samples *j* and *k* in eq. (3) can be rewritten as:

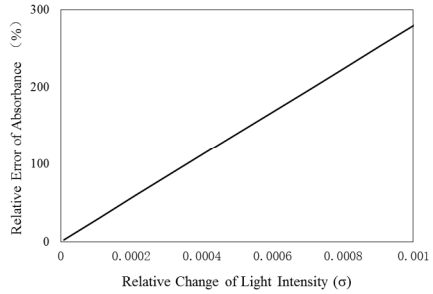
$$\Delta A(g) = \ln(1 + \frac{\Delta I}{I_0(t_{sj})}) + \varepsilon_g \cdot \Delta C_g \cdot l = \ln(1+\sigma) + \varepsilon_g \cdot \Delta C_g \cdot l \qquad (8)$$

Under the correction of corresponding reference sample, the absorbance difference between samples *j* and *k* in eq. (5) can be rewritten as:

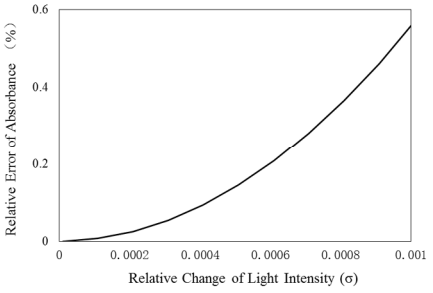
$$\Delta A(g) = \ln \frac{1}{1 + \frac{\Delta I}{I_{0bj}(t_{bj})}} \cdot (1 + \frac{\Delta I}{I_{0bk}(t_{bk})}) + \ln \frac{T_{bj}}{T_{bk}} + \varepsilon_g \cdot \Delta C_g \cdot l$$

$$= \ln \frac{1}{1+\sigma} \cdot \frac{1+3\sigma}{1+2\sigma} + \ln \frac{T_{bj}}{T_{bk}} + \varepsilon_g \cdot \Delta C_g \cdot l \qquad (9)$$

According to the research of Amerov *et al*., [17] the molar absorptivity of glucose, ε_g, at a wavelength of 1602.7 nm is approximately 6.44×10^{-5} $mM^{-1}mm^{-1}$ at 37℃. If the optical path length of the cell used in the experiment is 1 mm, a change of 100 mg/dL in the glucose concentration will lead to a change of 3.58×10^{-4} in absorbance, *i.e.*, $\varepsilon_g \cdot \Delta C_g \cdot l = 3.58\times10^{-4}$ in eqs. (8) and (9). The relative error of absorbance caused by the changes in the incident light intensity is shown in Fig. 1.



(a) Single-beam measurement.



(b) Single-beam measurement with reference correction.

Fig. 1. Relative errors of absorbance under various levels of light intensity drift.

As shown in Fig.1 (a), the relative error of absorbance increases linearly with the level of light drift. For the wavelength investigated, an increase of 0.0001 in σ leads to an increase of 30% in the relative absorbance error. For σ = 0.0001, the relative error in absorbance is approximately 27.93%. Therefore, a single-beam measurement usually only applies to the very short-term experiment with high SNR.

Fig. 1 (b) is the result of a single-beam measurement with reference correction, where the relative error of absorbance increases slowly with the light intensity drift. For the wavelength investigated, the relative error of absorbance is approximately 0.0056% with a relative drift level of 0.0001, which indicates that the influence of light drift on the absorbance measurement is efficiently reduced by the correction of the standard sample. However, once σ is larger than 0.001, the relative error of absorbance increases exponentially. Therefore, if there is a rather large change in light intensity in the measurement, such as when the time interval between the two samples is long or the two samples are measured on different days, the correction effect of this method will be quite limited.

Moreover, as the magnitude and direction of the drift cannot be controlled in practical experiments, there exist other inevitable error in absorbance, which will make the calculation of the absorbance more complicated.

2.2 Double-beam measurement

As mentioned above, another way to correct the light drift is a double-beam measurement, in which the incident light passes through the sample beam and the reference beam and is received by two detectors simultaneously. Here, the transmitted intensities of the sample beam and the reference beam are:

$$I_s = I_0(t) \cdot \eta_s \cdot e^{-(\sum_i \varepsilon_i c_i l + \varepsilon_g c_g l)} + \sigma_s \qquad (10)$$

$$I_b = I_0(t)\cdot\eta_b\cdot T_b + \sigma_b \quad (11)$$

where I_s and I_b are the transmitted intensities of the sample beam and the reference beam, respectively, η_s and η_b are the splitting properties of light on the sample beam and the reference beam, and σ_s and σ_b are the detector noises of the two paths during measurement. Generally, the thermal noise and shot noise are considered to be dominate. As thermal noise can be greatly reduced by working at a low-temperature, shot noise dominates for photomultipliers and photodiodes. Research shows that the bigger the dark current is, the larger the shot noise will be [18]. Currently, the dark current of detectors is approximately 5-50 nA, and a temperature controller is employed to keep the detectors working at low temperature to reduce the thermal noise. Therefore, in the following derivation, the noises from two detectors is ignored, that is, $\sigma_s = \sigma_b = 0$.

For a given wavelength λ, the splitting ratio of the reference beam and the sample beam is defined as $R(\lambda) = \eta_b / \eta_s$, which is constant at certain wavelengths. The splitting ratio can be determined by measuring the light intensity of two paths through the same sample or empty cells. Then, the equivalent absorbance of the sample beam can be expressed as:

$$A_e = \ln\frac{I_b(t)}{I_s(t)} = \ln\frac{I_0(t)\cdot\eta_b\cdot T_b}{I_0(t)\cdot\eta_s\cdot e^{-(\sum_i \varepsilon_i c_i l + \varepsilon_g c_g l)}} = \ln\frac{R(\lambda)\cdot T_b}{e^{-(\sum_i \varepsilon_i c_i l + \varepsilon_g c_g l)}} \quad (12)$$

As the light transmits through the sample beam and the reference beam simultaneously, the drift in the light source is eliminated by the double-beam design. Thus, the difference in the absorbance caused by the change of glucose concentration between sample *j* and sample *k* can be estimated as follows:

$$\Delta A(g) = A_{ej} - A_{ek} = \ln\frac{T_{bj}}{T_{bk}} + \varepsilon_g\cdot\Delta C_g\cdot l \quad (13)$$

If the standard sample in the reference beam is stable enough, that is, $T_{bj} = T_{bk}$, the absorbance difference induced by glucose is linear with the concentration difference between these two samples. Thus, this double-beam design is independent of the drift of the light source and the time interval between samples, which is useful for multi-day experiments.

The relative errors of absorbance induced by different instrumental drift level (mainly light source drift) under (1) a single-beam measurement, (2) a single-beam measurement with reference correction and (3) a double-beam measurement are listed in Table 1.

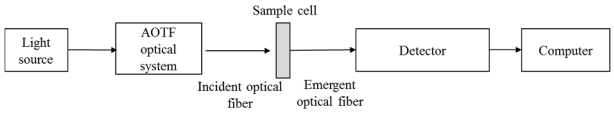
Table 1. Relative errors of absorbance for different levels of light source drift

Drift level	Relative errors of absorbance（%）		
	Single-beam measurement	Single-beam measurement with reference correction	Double-beam measurement
0.0001	27.93	0.0056	0
0.0005	142.42	0.14	0
0.001	279.19	0.56	0
Requirements	Extremely high SNR; very short measuring time;	High SNR; short time interval between sample and reference;	No requirement for SNR and time interval between samples;
Applications	Only short experiments	Single-day experiment	Single-day and multi-day experiment

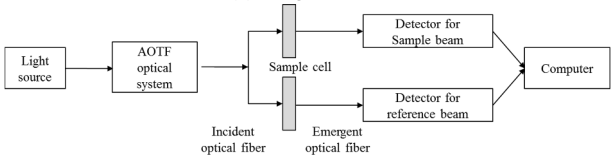
3. Materials and Methods

3.1 Instrumentation

A custom-developed NIR spectrometer is used, which is configured with a tungsten-halogen lamp of 100 W (PG64623, OSRAM, Germany), an acoustic-optic tunable filter (AOTF) (TEAF10-1.0-1.8-S, VFI-80-50-DDS-B1-C2-E, Brimrose Company, USA) and two InGaAs PIN photodiodes (G5851-21, Hamamatsu Photonics K.K., Japan). The spectral response range is 9090-5882 cm^{-1} (1100-1700 nm) with a resolution of 10 cm^{-1}. Quartz cells with path lengths of 1mm and an automatic sampling system with two peristaltic pumps are used in the experiments. Eight spectra for every sample were averaged to reduce the random noise. The schematics of single-beam and double-beam measurement systems are shown in Fig. 2(a) and (b). The only difference between Fig. 2(a) and (b) is the reference beam in double-beam system. Without it, the double-beam system can be used as a single-beam system.



(a) Single beam.



(b) Double beam.

Fig. 2 Schematic illustration of measurement system.

3.2 Reagents

Analytical-grade pure glucose powder and deionized water are used for the sample preparation. Deionized water is used as the standard sample for single-beam and double-beam system to correct the drift of the spectrometer. The three sample sets tested in this paper were as follows:

- Sample set 1:

20 samples with a glucose concentration of 200 mg/dL are prepared, coming from the same solution pool with a concentration of 200 mg/dL.

- Sample set 2:
  six samples with glucose concentrations of 0, 100, 200, 400, 500, 600 mg/dL are prepared.
- Sample set 3:
  (1) Samples of the training set: 25 samples with glucose concentration ranging from 0 to 700 mg/dL with intervals of 20 to 30 mg/dL are prepared.
  (2) Samples of the prediction sets: variable number of samples, *i.e.*, 6-30, with glucose concentrations ranging from 0 to 700 mg/dL with intervals of 20 to 200 mg/dL are prepared.
  Table 2 summarizes the number of samples and the detailed specifications for this part of the glucose data set.

Table 2. Specifications for glucose data sets

Day	Number of samples	Mean glucose concentration (mg/dL)	Standard deviation of glucose concentration (mg/dL)	Glucose concentration range (mg/dL)
1st	25	350.0	219.5	0-700
3rd	25	336.0	211.9	0-700
5th	28	347.3	216.3	0-700
7th	9	366.7	216.6	0-700
9th	6	400.0	248.9	100-700
11st	8	268.8	249.2	0-700
13rd	8	300.0	234.5	0-700
15th	10	250.0	202.8	0-600
17th	10	250.0	202.8	0-600
19th	30	335.0	217.2	0-700
21st	29	324.1	212.5	0-700
23rd	28	311.3	223.4	0-700
25th	9	283.3	259.8	0-700
27th	9	283.3	259.8	0-700
29th	20	181.5	155.4	0-500

The samples on the 1st day are used for calibration, and the samples on other days are used for prediction.

3.3 Experiments and methods

The whole experiment comprises three parts:

(1) A repetitive experiment for the purpose of evaluating the instrumental performance and investigating spectral change caused by time perturbation. Sample set 1 is applied in this experiment.

(2) A small sample and preliminary experiment to explore the absorbance spectra with a series of glucose concentrations. Sample set 2 is applied in this experiment.

(3) Multiple single-day experiments for PLS modeling. For the single-day experiments, transmitted spectra are collected within 5 hours. The multi-day experiments lasts approximately 30 days, with measurements conducted every two days. Sample set 3 is applied in this experiment.

The above experiments are performed by three measurement modes. For single-beam measurement, the transmitted spectra of glucose samples are collected. For a single-beam measurement with reference correction, the transmitted spectra of glucose samples and pure water are collected alternately. For double-beam measurements, the glucose samples and pure water are placed into the cells in the sample beam and the reference beam, respectively, and measured simultaneously.

Meanwhile, two analysis methods are tested in this article: two-dimensional correlation spectroscopy (2DCOS) and PLS regression analysis.

2DCOS, proposed by Noda, is a type of cross-correlation analysis method based on external perturbation and provides high spectral resolution and good interpretive ability for spectra [19-20].

As NIR spectroscopy analysis is an indirect technique, it is usually combined with a chemometrics method to extract the effective signals from complicated spectra and thus achieve the quantitative and qualitative analysis for the analyte of interest. PLS regression analysis is one of the most classic and useful multivariate linear methods to correlate the spectral variance with that of physical or chemical information. In this paper, the PLS calibration model is built and validated by leave-one-out cross-validation. The root mean square error of prediction (RMSEP) and the correlation coefficient (R) are calculated to evaluate the model:

$$RMSEP = \sqrt{\frac{\sum_{i=1}^{n}(\hat{y}_i - y_i)^2}{n-1}} \quad (14)$$

$$R = \sqrt{\frac{\sum_{i=1}^{n}(\hat{y}_i - y_i)^2}{\sum_{i=1}^{n}(\bar{\hat{y}}_i - y_i)^2}} \quad (15)$$

where y_i is the actual concentration value, $\hat{y}_i$ is the predicted concentration value, $\bar{\hat{y}}_i$ is the average value of $\hat{y}_i$, and n represents the number of samples involved in the modelling.

4. Results and Discussion

4.1 Instrument performance evaluation

As the double-beam measurement system can be used in single-beam mode without the reference beam, it is evaluated under a different mode.

The ratio of the reference beam to the sample beam, measured for the water sample, is shown in Fig. 3. We find that, the intensity of the reference beam is slightly higher than that of the sample path, especially in the region of 1400 nm to 1600 nm. In the double-beam measurement system, the incident light from the light source is divided into two beams by a custom-designed fiber, which consists of a large number of single-mode fibers. Restricted by the manufacturing technology of fibers, it has been very hard to ensure the congruence of two beams. Even though there exists variation in intensities, the changes in the two beams are synchronized and homologous because they originate from the same light source, so this difference in intensity will not affect the results of the double-beam measurement.

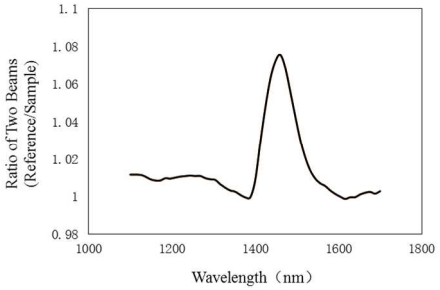


Fig. 3 Spectral intensity ratio of the double-beam system.

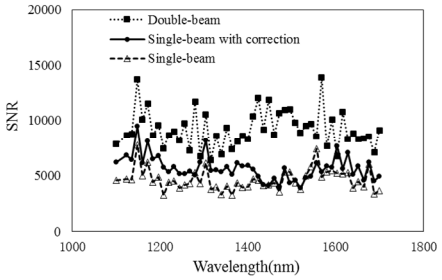
The 200 mg/dL aqueous glucose solution and pure water sample are used to evaluate the SNR of the spectrometer in a single experiment.

The SNR of 1 minute under different measurement modes is shown in Fig. 4(a); it is approximately 9000:1, 6000:1 and 5000:1 for the double-beam measurement, single-beam measurement with reference correction, and single-beam measurement without reference correction. The experiments are repeated 20 times, which takes approximately 3 hours, and the SNR result is shown in Fig. 4(b). Here, some conclusion can be made:

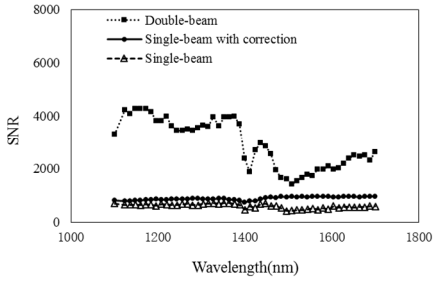
(1) The SNR decreases significantly with increasing test time. When the test time increases from 1 minute to 3 hours, the SNR under single-beam measurement with correction decreases from 6000:1 and ~1000:1, while the SNR under double-beam measurement decreases from 9000:1 to 3000:1(1100-1400 nm) or even 2000:1(1400-1700 nm). Specifically, the different reductions corresponding to respective spectral ranges are mainly attributed to two factors: ① Influenced by the absorption of water and glucose, the spectral intensities in the 1400-1700 nm region are relatively weak compared with those of other wavelengths. For the same level of instrumental noise, the weaker the useful signal is, the greater the influence of the noise will be, and this will ultimately result in an undesirable SNR at wavelengths with low signal. ② The absorption is likely to increase because of OH^- in the fiber, which also leads to a low signal intensity.

(2) Reference sample subtraction is an effective way to enhance the stability of measurement. For the single-beam measurement, the SNR is improved slightly by the correction of the reference sample, where the 3-hour SNR in the 1100-1400 nm and 1400-1700 nm regions increases from 600:1 to 800:1 and 500:1 to 1000:1, respectively.

(3) The SNR for double-beam measurements is higher than that for single-beam measurements in both the 1-minute and the 3-hour tests, which means that a double-beam measurement is effective at eliminating the light drift, even in the long time experiments.



(a) 1-minute test.



(b) 3-hour test.

Fig. 4 SNR under different measurement modes.

To further explain the reason for high SNR in the double-beam measurement, 2DCOS is applied to investigate the influence of instrumental drift. Considering the spectra of the 200 mg/dL aqueous glucose solutions chronologically, the synchronous 2D correlation spectra under the perturbation of time in different measurement modes are calculated, as shown in Fig. 5(a), (b) and (c). The auto-peaks appearing at diagonal positions in the synchronous map represent the overall extent of spectral intensity variation observed at the specific spectral variable during the period of observation. The corresponding slice spectra in the diagonal are presented in Fig. 5(d).

As the spectra of the same sample are investigated, all of the spectral variations due to changes in the measuring time are from the status change of the spectrometer. In the synchronous correlation spectra contour maps shown in Fig. 5(a) and 5(b), there is little difference between the result of direct measurement and single-beam measurement with correction. However, difference can be observed between Fig. 5(a) and (b) and Fig. 5(c). The detailed information about the auto-peaks shown in Fig. 5(d) reveals their differences clearly. Two relatively obvious auto-peaks (1400 nm and 1496 nm) can be observed in the slice spectra of direct measurement and single-beam with correction, and the intensity in the longer wavelength is larger. This result indicates that the longer wavelength

is more sensitive to the status change of the spectrometer or the outside conditions. The influence of water causes this phenomenon. An aqueous glucose concentration of 200 mg/dL is used for experiments in which the proportion of water is extremely high. As mentioned above, the absorption effect of water increases gradually above 1400 nm; consequently, during an experiment with a long time period, the spectral signal is more likely to be influenced by outside factors such as instrument drift, typically in the wavelength band where there is a strong absorption.

In addition, the intensities of slice spectra decrease after reference correction, especially for longer wavelengths. Moreover, the intensity of slice spectra for double-beam measurement is lowest and remains at the same level under the wavelengths investigated. All of these results further explain the SNR obtained in Fig. 4 under different measurement mode and verify that using a reference sample can eliminate some light source drift because the double-beam design has best performance in removing the time-dependent spectral variations.

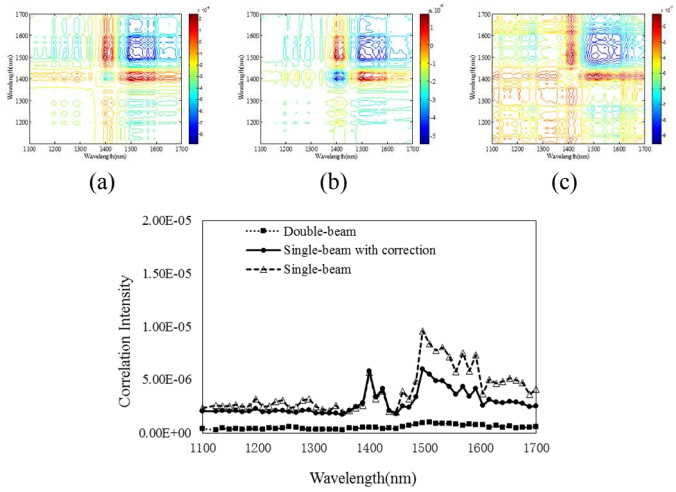
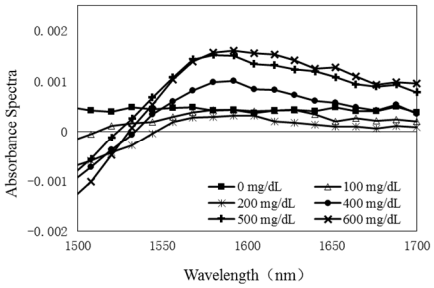
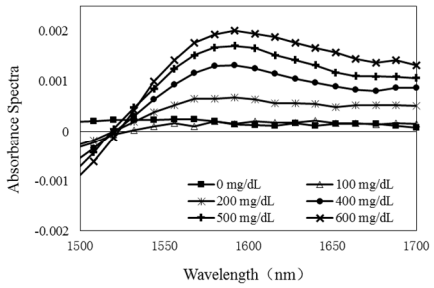


Fig. 5 Synchronous 2D correlation spectra of an aqueous glucose solution (200 mg/dL) under the perturbation of measuring time in different measurement modes: (a) single-beam measurement, (b) single-beam measurement with reference correction, (c) double-beam measurement, and (d) their slice spectra on the diagonal.

According to previous studies, the first-order band of glucose exists over the region 1500-1800 nm with a central wavelength of 1587-1600 nm [21]. A series of absorbance difference spectra of sample set 1 obtained using single-beam measurement with pure water correction or double-beam measurement are shown in Fig. 6 (a) and (b), respectively. Obviously, the characteristic absorption peaks of glucose can be easily observed both in single-beam and double-beam measurement. There is a slight overlap in the absorbance spectra under single-beam measurement, which leads to the absorbance not arranging in order of glucose concentrations. It indicates that the variation in the spectra caused by the light source drift interferes with the change in the glucose signals to some degree. However, the absorbance under the double-beam measurement can be easily recognized according to their glucose concentrations.



(a) Single-beam measurement with water correction.



(b) Double-beam measurement.

Fig. 6 Absorbance difference spectra of glucose solutions.

4.2 Single day experiment

The transmitted spectra of 25 samples with different glucose concentrations collected within 5 hours on the first day are used to build the PLS model of glucose. The prediction results are listed in Table 3, where the model based on single-beam measurement performs worst. After the correction using a pure water sample, the RMSEP is reduced by approximately 33.77%. The RMSEP of the model based on a double-beam measurement is 13.21 mg/dL, which is reduced by 31.31% in comparison with a single-beam measurement with reference correction. Obviously, the calibration model based on a double-beam measurement performs best. These results are consistent with the SNR results test.

Table 3. Modeling results of different measurement modes

Method	Number of latent variable	Calibration		Prediction	
		RMSEC (mg/dL)	R	RMSEP (mg/dL)	R
Single-beam measurement	4	20.02	0.987	29.04	0.976
Single-beam measurement with reference	2	15.71	0.992	19.24	0.990

correction					
Double-beam measurement	2	10.92	0.996	13.21	0.995

Moreover, the number of the principal components, *i.e.*, the latent variable number to build the calibration model, varies for different measurement modes, decreasing from four when using direct measurement to two when using other methods with correction. One possible reason is that the spectral variation from light drift has been eliminated by the correction of the reference sample or the beam. As mentioned above, the reference sample can actually monitor and compensate for the light intensity drift if the short-term SNR of the instrument is high and the sampling interval between the sample and the reference is short. The light source drift is eliminated by the reference beam under the condition of low detector noise. Therefore, the reference sample correction and the double-beam design can reduce the drift and reduce the dimension of the spectral matrix.

4.3 Multi-day experiments within 30 days

To develop a reliable quantitative analysis model, multi-day experiments are usually required, especially for noninvasive blood glucose concentration sensing, where the glucose content in blood is low and the fluctuations relative to the physiological and environmental background are complex and uncontrolled. For the calibration model based on multi-day experiments, obstacles for an accurate model are the light source drift with the time, the influence of physiological activities and other unpredicted variation. As a result, the reliability of different measurement modes in multi-day experiments must be investigated.

Fig. 7 shows the signal intensity of pure water solutions within 30 days, which were collected every two days. Obviously, the spectra on different days overlap with each other because the whole experimental process lasted for almost one month, which meant rebooting the spectrometer each time we resumed the experiment.

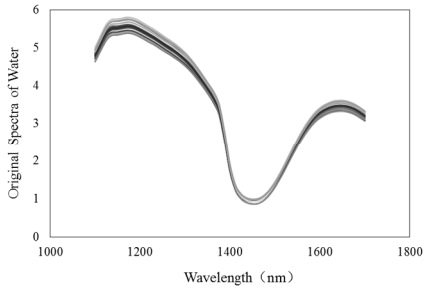


Fig. 7. Transmitted spectra of water within 30 days over 1100-1700 nm.

The spectra from the first day are then used to build the glucose model to predict the data on the other days. As the single-beam measurement does not apply to a long-term experiment, only the single-beam measurement with reference correction and the double-beam measurement are conducted, and the prediction result are shown in Fig. 8.

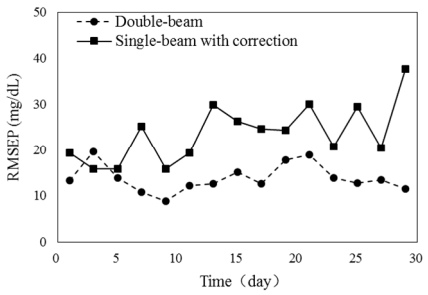


Fig. 8 Predicted results for multi-day experiment by the calibration models from the first day.

As seen in Fig. 8, for the single-beam measurement with reference correction, the predictive ability varies with the time because of the fluctuations of the instrumental light source, and the general trend increases with the experiment date. For example, the RMSEP for the data on the 3rd day is approximately 15.73 mg/dL, while it increases to 37.69 mg/dL for the data on the 29th day. The average RMSEP is 23.59 mg/dL. The factors contributing to this phenomenon are that during multi-day experiments the spectrum of the light source itself will unavoidably change, and, importantly, the changes at different wavelengths of the spectrum are not identical. Consequently, the absorbance calculated based on that spectrum will be different from that calculated from the calibration set. Moreover, the drift of the light source occurs during every single experiment, with unpredictable magnitude and tendency, which will also lead to changes in the absorbance. As a result, errors will emerge when we use the mathematical relationship between absorbance and concentration in the calibration model to predict unknown samples.

By contrast, the calibration models based on the double-beam measurement perform quite stably, and most of the RMSEP values are less than 15 mg/dL except those on the 3rd, 19th and 21st days. Obviously, the double-beam measurement has better predictive ability, with an average RMSEP of 13.76 mg/dL, which is 41.67% less than that of the single-beam measurement with reference correction.

5. Conclusion

In the NIR spectroscopy technique, the background changes of the optical system can lead to a significant effect on the extraction of a useful signal. In this paper, the basic principles of single-beam measurement, single-beam measurement with reference correction and double-beam measurement with double detectors are analyzed. Their effect on eliminating the influence of light source drift is also investigated under the condition that the drift of the light source over time is dominant and the noise of the detector can be ignored. The requirements for the instrument and the application of different measurement modes are summarized. Theoretically, single
-beam measurement highly depends on the SNR of the instrument because it has no processing for light drift; the single-beam measurement with reference correction can eliminate the light drift under the condition of a short time interval between analysis of the

sample and the standard; the double-beam measurement has no special requirements for SNR or the time interval between samples. Finally, the ability of the three measurement modes to reduce the influence of light drift is investigated by single-day and multi-day aqueous glucose experiments.

The results show the following: (1) Among these three measurement modes, the double-beam design best eliminates the time-dependent variations, and the SNR of a single-beam measurement can be improved by correcting with a reference sample. These results are also verified by the 2D correlation spectral while varying the measurement time. (2) For a single-day experiment, the PLS model based on the double-beam design is most accurate with an RMSEP of 13.21 mg/dL, which is 31.31% less than that of a single-beam measurement with reference correction. (3) For multi-day experiments, the predictive ability of the PLS model based on a double-beam measurement performs stably with an average RMSEP of 13.76 mg/dL, which is 41.67% less than that of a single-beam measurement with reference correction, which has a predictive ability that varies with the experiment date. Therefore, it can be concluded that both the reference sample correction and the reference beam design can reduce the light drift effectively; by contrast, the double-beam design is more effective, especially in the multi-day or long-term experiments.

The single-beam measurement with reference correction and double-beam measurement can eliminate the light drift to a certain degree, and satisfactory results have been achieved in an aqueous glucose experiment. However, it is worth noting that, all of these theoretical derivations and experiments are based on the drift of the light source over time being dominant and the standard sample having similar optical characteristics to the sample of interest. When those designs are applied to *in vivo* experiments, there are still problems that need to be considered. For example, for non-invasive glucose concentration sensing using NIR spectroscopy, the variations due to the body's physiological activities, such as temperature or tremble, may be larger than that from the instrument. However, these physiological activities are usually strictly uncontrolled and their influences are difficult to quantify. Furthermore, it is difficult to find a reference material that has similar optical properties to the human body. Therefore, the single-beam measurement with reference correction and the double-beam measurement have some limitations in *in vivo* experiments, and some improvements are needed. Our group is now trying to use the spectrum of a special wavelength or certain source-detector distance, for which the spectra are insensitive to the variation in glucose concentration, as the internal reference to eliminate the complicated variations from physiological activities and instrumental drift *in vivo* [22-23]. Further study is currently under way.

6. Acknowledgements

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